THE RELATION OF RHYTHMS AND ENDOMIXIS, THEIR PERIODICITY AND SYNCHRONISM IN PARAMECIUM AURELIA.

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Periodic nuclear reorganization or endomixis in *Paramecium*, noted by Hertwig ('88–'89) and since then by many other investigators, and described in detail by Woodruff and Erdmann ('14), is of great interest because of its bearing on questions of parthenogenesis, immortality and nuclear functions in Protozoa primarily, and secondarily in organisms in general. In a series of recent papers Woodruff and Erdmann ('14, '16) and Woodruff ('17, *a* and *b*) have advocated the view that it is a sine qua non in the life of *Paramecium*, that it is a rejuvenating process analogous to the similar process in conjugation, and that it usually occurs at the low point in the division rate between two "rhythms," the time interval of which is a fixed quantity inherent in the species and which cannot be modified by the external environment.

Kasanzeff ('01), Popoff ('09), Sun ('12) and myself (Young, '17) have shown that external agents may serve to induce endomixis, and my own experiments show that the frequency of its occurrence may be increased in this way. In my paper (*l. c.*, p. 52) I have stated that "It is more or less cyclical in character and probably has a rejuvenating function. It is not however a necessary accompaniment of temporary depressions and may not be followed by rejuvenation." I have pointed out several cases of the occurrence of low division points without endomixis in Woodruff's and Erdmann's experiments, as well as in my own. I have also indicated one case in their experiments and one in my own in which the process was associated with a rising, rather than a falling division rate; and have shown that in some instances endomixis is not followed by rejuvenation.

In the graphs of Woodruff's recent papers ('17, *a* and *b*) are shown several instances of low division points without endomixis, viz., in ('17*a*), Fig. 2, periods 1 and 12; Fig. 3, periods 1, and 5 and 6; Fig. 4, periods 2 and 11; Fig. 5, period 19; and in ('17*b*) Fig. 1, A, period 22; AE, periods 2 and 13; O, period 11; B, periods 22 and 23, and 27; Fig. 2, A_T, periods 3, 19 and 25; AE_T, periods 12, 17, 19, 25 and 27; O_T period 12 and B_T period 23, etc.

In Fig. 4 ('17*a*) are shown two low points without endomixis which appear to me essentially similar to those at which this process occurred (cf. per. 2 and 11). If the latter mark the division point between rhythms, why not the former? In this connection I wish to call attention to the author's words on p. 55: "During this time nine rhythms occurred at each of which endomixis was observed. *It is hoped that readers who may possess doubts concerning rhythms and endomixis will carefully consider this graph.*[1]

Regarding periods 19 and 25, A_T, Fig. 2 ('17*b*) and similar periods elsewhere Woodruff says (p. 446): "A second point of importance is that the 'rhythms' in the division rate are to a certain extent independent of endomixis—that is of the definite series of nuclear phenomena—because the rhythms persist for a while in the absence of the morphological changes. . . . So, it seems to be a more or less academic question whether rhythms and endomixis are independent. Certainly the rhythms occur for a while without endomixis in *sensu stricto*—but *in all such cases*[2] the culture has died before very long." While this statement applies to *some* of Woodruff's experiments it by no means applies to all. Many of the cases cited above, and many other cases shown in his graphs which I have not cited, besides several cases in my own experiments noted by me (*l. c.*, p. 47) disprove it. See for example his own statement (*l. c.*, p. 446) referring to subculture B_T, Fig. 2, "From the character of the curve it would be expected at period 23, but it was not observed."

This leads us to the question of the meaning of "rhythm" and of the influence of the graph in determining the same. Accepting Woodruff's definition of the former as " a minor periodic rise and

[1] Italics mine.

[2] Italics mine.

fall of the fission rate, due to some unknown factor in cell metabbolism, from which recovery is autonomous" ('05, p. 604), we must consider the then "unknown factor," which virtually makes "rhythm" synonymous with inter-endomictic period, a sense in which the term "endomixis was *not* used . . . when first employed by Woodruff and Erdmann" (Woodruff, '17*b*, p. 447). But if we do not consider this factor in defining "rhythm," its definition becomes a wholly arbitrary matter dependent upon the character of the graph employed, which as Woodruff and Erdmann ('14, p. 447) have shown is at best an artificial means of representing the history of the race in question, and which tends to emphasize or obscure the rhythms as the average period is shortened or lengthened.

While cessation of endomixis was followed by death in two of Woodruff's experiments (A_T and AE_T, '17*b*), both my own and Woodruff's experiments show many cases in which the process occurred shortly before the death of the race in question—see my Figs. II, VIII, VIII*a*, X*a*, etc., and Woodruff's ('17*b*) Figs. 1, O, and 8, B_8 and M_8. It is of course entirely possible that death in the former cases resulted from cessation of endomixis, and in the latter from some other cause, but on this question the experiments throw no certain light.

Woodruff's recent work has further disclosed several additional cases of the exceptional occurrence of endomixis at a high, or rising, rather than a low, or falling point in the division rate. I have previously noted two of these exceptions (*l. c.*, p. 47) one in my own experiments and one in those of Woodruff and Erdmann (1914), to which may be added here the occurrence of the process at periods 68 O, 81 B, Fig. 7; 61 A_8, 82 and 86 O_8, 70 B_8, 70 and 82 M_8, Fig. 8 and 76 M, Fig. 12.

In most of Woodruff's graphs the variations are too irregular or the period too short to show any regular rise and fall in the division rate over considerable periods, but in one case (*A*, Fig. 9, p. 455, 17*b*) such alternate periods are clearly shown, without however any evident correspondence between endomixis and the periods of low and high division rate. An endomixis is as liable to occur at a high point as at a low point on the curve, neglecting the minor fluctuations of a few tenths of a division which mark

the intermediate points of the "rhythms" in this curve. The same is true of other curves in which an alternate rise and fall in the division rate over long periods is not evident. Take for example the graph of line *M* in Fig. 12 (*l. c.*, p. 458). Here there is no relation whatever between absolute division rate and endomixis, the process occurring four times during division periods of 2 + per day and five times when the division rate was 2 — per day, the only relation between endomixis and division rates being evident in the minor fluctuations or "rhythms."

In view of these results, I cannot find any constant correlation between endomixis and division rate in *Paramecium*.

Why then is it that endomixis is *usually*, though not always, accompanied by a temporary reduction in the division rate? I believe the latter to be consequent to the former. During endomixis profound chemical and physical changes are taking place in the cell, of whose nature we are at present ignorant, but which must necessarily exercise a great influence on metabolism and growth, and would naturally tend to temporarily diminish the division rate. Those exceptional cases in which endomixis occurs at a high, or rising point in the division rate may perhaps be attributed to some extrinsic stimulus in the culture medium, tending to reverse the usual sequence of events.

In his various papers on the subject of "rhythms" in Protozoa, Woodruff maintains a more or less regular periodicity in their occurrence (25–30 days or 40–50 generations in *Paramecium aurelia* and 50–60 days or 80–100 generations in *P. caudatum*). In his most recent paper ('17*b*, p. 461) he states that "General changes in the environment of the animals . . . do not permanently modify the length of the rhythm or the time between successive endomicitic periods which is characteristic of the species." But (p. 462) "it has been found that the generation periodicity . . . may be modified to a considerable degree by the culture conditions which lower the division rate. In other words, the rhythm appears to be more susceptible of modification in regard to generations than time. As has been previously noted, this is a surprising result, since a profound reorganization process such as endomixis must be closely related to the general metabolism of the cell and this is expressed to a large extent in growth and reproduction."

With the second of these conclusions I am wholly in accord,[1] agreeing as it does with the results of my own work (*l. c.*). The first is not however in accord with my experiments and cannot I believe be verified by a critical reading of Woodruff's own results. That the periodicity of the rhythms is subject to greater variation than he admits may be deduced from his own data. In order to show this I have compiled a table based upon the graphs and tables of several of his own and Miss Erdmann's recent papers, the exact source of the data being indicated in every case.

In this table are shown the number of five-day periods, generations and variation percentages of the rhythms in various lines of *Paramecium aurelia*, based on data the source of which is indi-

1.	2.	3.	4.	5.	6.	7.	8.	9.
5	4.7	+ 6	64	47	43.5	+ 8	27	W & E ('14) line VI, Table 1 and text fig. 16.
3		−36		36		−17		
5		+ 6		43		− 1		
6		+28		48		+10		
				79	59.5	+33	66	*idem*, line VI*h*, Table 1.
				40		−33		
8[4]	6.5	+23	76	75[2]	62.5	+20	48	*idem*, line III, Table 2 and text fig. 17.
4[4]		−38		49[2]		−22		
5		−23		48		−23		
9		+38		78		+25		
4	4.7	−15	85	53	55.9	− 5	104	W ('17*a*), fig. 4.
6		+28		80		+43		
5		+ 6		56		00		
6		+28		60		+ 7		
6		+28		70		+25		
2		−57		22		−61		
4		−15		47		−16		
5		+ 6		59		+ 6		
2	5	−60	100	27	47.8	−44	124	*idem*, fig. 5.
7		+40		86		+80		
4[3]		−20		30[3]		−37		
7[3]		+40		53[3]		+11		
5		00		43		−10		

[1] Using "rhythm" as synonymous with inter-endomictic period.

[2] According to Woodruff's data there is a little uncertainty regarding the occurrence of endomixis at generation 4140 in line III. I have based my data on the assumption of its occurrence at that time which he states was "almost positive" (*l. c.*, p. 470).

[3] Uncertainty exists in Woodruff's data regarding the occurrence of endomixis at period 14. My table is based on the assumption of its occurrence there, which he considers very probable judging by "the character of the curve" (*l. c.*, p. 56).

1.	2.	3.	4.	5.	6.	7.	8.	9.
5	5.5	− 9	72	34	42.4	−20	102	W ('17*b*) figs. 1 and 7,
6		+ 9		54		+27		line A.
6		+ 9		60		+41		
7		+27		69		+63		
4		−27		45		+ 6		
5		− 9		43		+ 1		
7		+27		31		−27		
5		− 9		33		−22		
8		+45		45		+ 6		
4		−27		26		−39		
4		−27		26		−39		
5	5.2	− 4	38	53	54.5	− 3	53	*idem*, line O.
6		+15		71		+30		
5		− 4		60		+11		
4[1]		−23		50[6]		− 8		
6[1]		+15		47[6]		−14		
5		− 4		42		−23		
4	5	−20	80	78	55	+42	97	*idem*, fig. 7, line B.
7		+40		59		+ 7		
6		+20		58		+ 5		
3		−40		25		−55		
2	4	−50	75	10	12	−17	50	*idem*, fig. 8, line A_s.
4		00		15		+25		
5		+25		14		+17		
5		+25		9		−25		
5	4.7	+ 6	21	36	30	+20	33	*idem*, line O_s.
5		+ 6		28		− 7		
4		−15		26		−13		
3	4	−25	100	29	31.6	− 8	82	*idem*, line M_s.
2		−50		17		−46		
6		+50		37		+17		
5		+25		43		+36		
4		00		32		+ 1		

[1] I have considered the two endomixes shown on the graph at periods 61 and 63 as a single event, which I infer to be the case from the table on page 452.

cated as follows: W = Woodruff, W & E = Woodruff and Erdmann, with the date of publication following the author's initials in each case.

Columns 1 and 5 show the number of five-day periods and generations respectively, columns 2 and 6 the average of each, columns 3 and 7 the variation percentages based on the average, columns 4 and 8 maximum differences of the latter, and column 9 the source of the data. In every case "rhythm" is the same as inter-endomictic period.

The above data are sufficient I think to refute Woodruff's

claim as to rhythmical constancy in *Paramecium*. Certainly variations of from fifteen to twenty-five days in periods never exceeding forty-five days are scarcely in accord with his statement ". . . that once established the rhythmic period characteristic of the species is maintained within rather narrow limits" ('17*b*, p. 452).

In both his joint paper with Erdmann ('14) and his most recent one ('17*b*) Woodruff cites Tables 1, 2 and 3 ('14, pp. 462–463) as evidence of synchronism of endomixis. Again a careful reading of these tables fails to support his contention. Of twenty-one endomixes shown in Table 1 for example, fifteen show close or fairly close synchronism, but of these four occurred in lines which had but recently been branched off from the parent lines, and hence had not yet had opportunity to vary to any considerable degree from the condition of the latter. I refer to the endomixis at generation 4101 in line VIC and to those at about generation 4180 in VId, e, and g. If we reject these as proving nothing one way or the other, we find that out of seventeen endomixes eleven support Woodruff's claim, while six fail to do so. The exceptions in other words are too numerous to "prove the rule." Of especial interest are those lines which, while only recently started, show a marked divergence from the parent stock, showing that the supposed synchronism may be disturbed within a very few generations (VIb–4092, VIf–4189). This fact has been noted by Woodruff and Erdmann ('14, p. 461), but passed by with the remark that it " . . . has no significance from the standpoint of the synchronal appearance of the process in sister *lines* . . ." Further evidences of lack of synchronism are afforded by line IIIa, generation 4271, Table 2 and line V, generation 4115, Table 3.

Synchronism is better shown in *P. caudatum* (cf. Table 1, p. 69, Woodruff and Erdmann, '16) but even here there are a few exceptions (cf. lines I and III, generations 1–9, column 1). Other exceptions are shown in line Ica, generations 180–270, column 3, and in line Ie generations 270 to 450, columns 4 and 5, in Table 3 (*l. c.*, p. 71).

Regarding the synchronism of the same races of *Paramecium* when bred in different environments, Woodruff's table ('17*b*,

p. 455) shows that of seventeen endomixes in the four pairs of cultures to be compared,[1] the synchronism was exact in only six cases, approximately exact (*i. e.*, within one five-day period) in six and inexact (differing by at least two five-day periods, which in a total interval of never more than seven and seldom more than five such periods is considerable) in five. These facts, taken from Woodruff's own data, hardly seem to prove his claim regarding the maintenance of rhythmical constancy and synchronism in *Paramecium* in either the same or different environments.

Were this negative evidence the only refutation of his contention however I should hesitate to urge it too strongly. But I have positive evidence from my own experiments leading to directly opposite conclusions.

The graphs in my previous paper (*l. c.*) show numerous cases of irregularity in the time period of endomixis. In experiment 1 (Figs. 1 and 1*a*) for example line I shows a fairly regular endomictic periodicity with an average interval of eighteen days,[2] while line Ia, branched off from I on 5/8 was carried for a period of forty-two days without any endomixis whatever. Here the disturbance of the period is obviously due to changed environment; I being carried for the most part in stale, and Ia in fresh culture media. Experiment 2 shows that, whereas the first three endomicitic periods in line II, branched from I on 2/8, agree fairly well with the corresponding periods in I, following the endomixis on 3/5 no others occurred until 5/13, a period of sixty-nine days. This change in period is probably traceable in part to changed environment, as suggested in my previous paper (*l. c.*, p. 39). It is probable however that some factor inherent in the cell itself is also in part responsible since the line did not undergo the *urea* treatment until 4/21, forty-seven days after the last previous endomixis occurred. Whatever the cause however the experiment shows that marked variations in endomictic periods do occur.

Both lines IIa and III which are derivatives of line II, while showing essentially the same periodicity with respect to each

[1] Not counting those at period 59 which may have been induced by the sudden change to the stale culture medium.

[2] Omitting the doubtful record on 3/16 cf. footnote 5, p. 39 (*l. c.*).

other, show a periodicity differing from both the earlier and later period in the parent line II; while lines IV and VI, derived from II on 4/13 and 4/15 respectively, show periodicities differing from each other and from those of all three preceding lines (II, IIa and III). In line XII marked variations in periodicity are shown.

Since these experiments were not designed to test the periodicity of endomixis it is difficult in most of the above cases to determine the cause of the variations noted, whether extrinsic (environmental) or intrinsic. In the following experiments however we have very clear evidence of the influence of environment, in this case temperature, in altering the endomictic period. Comparing the length of period in lines XIIa and b with that in the parent line XII for example it is seen that increasing the temperature reduced not only the generation period, but also the time period, from an average of twenty-five days in XII to one of seven in XIIa and of twelve in XIIb. Even more striking evidence of the same character is furnished by experiments 14, 15 and 16.

Notwithstanding Woodruff's emphasis upon the constancy of the time period of endomixis, in contrast to the generation period, which latter was markedly affected by changed environment; it is reasonable to assume that the latter is the more important, indicating as it does the amount of growth and hence the metabolic activity of any period. He himself says (*l. c.*, p. 458), "This is a most surprising result, because such profound reorganization phenomena as are involved in endomixis must have a more or less definite relation to the physiological activity of the protoplasm, the best criterion of which is generally considered to be growth and reproduction as indicated by the division rate. More experiments obviously are needed to resolve this 'time factor' into its significant elements." In the absence of further experiments and in the light of the contradictory results of my own experiments and of many of those of Woodruff himself as analyzed above, I would suggest that the time synchronism, when present, is a result of generation synchronism; and that in those cases in which the latter, but not the former was modified by environment, the constancy of the former in those cases in which it was constant or approximately so (twelve out of seven-

teen) was due to chance, and not to any inherent constancy in the periodicity itself.

Undoubtedly there is more or less regularity in endomixis, just as in any other physiological process (cell division, reproduction, respiration, excretion, etc.); but to prove that this has a fixed time periodicity, which, unlike that of other physiological processes, cannot be modified by environment, will require much stronger evidence than Woodruff has as yet presented. His own data are contradictory and my results point very strongly to an opposite conclusion.

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[1] Not seen by me.